



130 135 140  
 Thr Cys Ile Ile Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly  
 ACG TGC ATC ATC AAT GGG GTG TGC TGG ACT GTC TAC CAC GGG GCC GGA  
 TGC ACG TAG TAG TTA CCC CAC ACG ACC TGA CAG ATG GTG CCC CGG CCT  
 145 150 155  
 Thr Arg Thr Ile Ala Ser Pro Lys Gly Pro Val Ile Gln Met Tyr Thr  
 ACG AGG ACC ATC GCG TCA CCC AAG GGT CCT GTC ATC CAG ATG TAT ACC  
 TGC TCC TGG TAG CGC AGT GGG TTC CCA GGA CAG TAG GTC TAC ATA TGG  
 160 165 170  
 Asn Val Asp Gln Asp Leu Val Gly Trp Pro Ala Ser Gln Gly Thr Arg  
 AAT GTA GAC CAA GAC CTT GTG GGC TGG CCC GCT TCG CAA GGT ACC CGC  
 TTA CAT CTG GTT CTG GAA CAC CCG ACC GGG CGA AGC GTT CCA TGG GCG  
 175 180 185 190  
 Ser Leu Thr Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr  
 TCA TTG ACA CCC TGC ACT TGC GGC TCC TCG GAC CTT TAC CTG GTC ACG  
 AGT AAC TGT GGG ACG TGA ACG CCG AGG AGC CTG GAA ATG GAC CAG TGC  
 195 200 205  
 Arg His Ala Asp Val Ile Pro Val Arg Arg Arg Gly Asp Ser Arg Gly  
 AGG CAC GCC GAT GTC ATT CCC GTG CGC CGG CGG GGT GAT AGC AGG GGC  
 TCC GTG CGG CTA CAG TAA GGG CAC GCG GCC GCC CCA CTA TCG TCC CCG  
 ↑  
 NaeI  
 210 215 220  
 Ser Leu Leu Ser Pro Arg Pro Ile Ser Tyr Leu Lys Gly Ser Ser Gly  
 AGC CTG CTG TCG CCC CGG CCC ATT TCC TAC TTG AAA GGC TCC TCG GGG  
 TCG GAC GAC AGC GGG GCC GGG TAA AGG ATG AAC TTT CCG AGG AGC CCC  
 225 230 235  
 Gly Pro Leu Leu Cys Pro Ala Gly His Ala Val Gly Ile Phe Arg Ala  
 GGT CCG CTG TTG TGC CCC GCG GGG CAC GCC GTG GGC ATA TTT AGG GCC  
 CCA GGC GAC AAC ACG GGG CGC CCC GTG CGG CAC CCG TAT AAA TCC CGG  
 240 245 250  
 Ala Val Cys Thr Arg Gly Val Ala Lys Ala Val Asp Phe Ile Pro Val  
 GCG GTG TGC ACC CGT GGA GTG GCT AAG GCG GTG GAC TTT ATC CCT GTG  
 CGC CAC ACG TGG GCA CCT CAC CGA TTC CGC CAC CTG AAA TAG GGA CAC

Figure 1 (continued)



385 390 395  
 Leu Gly Ile Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg  
 TTG GGC ATT GGC ACT GTC CTT GAC CAA GCA GAG ACT GCG GGG GCG AGA  
 AAC CCG TAA CCG TGA CAG GAA CTG GTT CGT CTC TGA CGC CCC CGC TCT

400 405 410  
 Leu Val Val Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr Val Pro  
 CTG GTT GTG CTC GCC ACC GCC ACC CCT CCG GGC TCC GTC ACT GTG CCC  
 GAC CAA CAC GAG CGG TGG CGG TGG GGA GGC CCG AGG CAG TGA CAC GGG

415 420 425 430  
 His Pro Asn Ile Glu Glu Val Ala Leu Ser Thr Thr Gly Glu Ile Pro  
 CAT CCC AAC ATC GAG GAG GTT GCT CTG TCC ACC ACC GGA GAG ATC CCT  
 GTA GGG TTG TAG CTC CTC CAA CGA GAC AGG TGG TGG CCT CTC TAG GGA

435 440 445  
 Phe Tyr Gly Lys Ala Ile Pro Leu Glu Val Ile Lys Gly Gly Arg His  
 TTT TAC GGC AAG GCT ATC CCC CTC GAA GTA ATC AAG GGG GGG AGA CAT  
 AAA ATG CCG TTC CGA TAG GGG GAG CTT CAT TAG TTC CCC CCC TCT GTA

450 455 460  
 Leu Ile Phe Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys  
 CTC ATC TTC TGT CAT TCA AAG AAG AAG TGC GAC GAA CTC GCC GCA AAG  
 GAG TAG AAG ACA GTA AGT TTC TTC TTC ACG CTG CTT GAG CGG CGT TTC

465 470 475  
 Leu Val Ala Leu Gly Ile Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp  
 CTG GTC GCA TTG GGC ATC AAT GCC GTG GCC TAC TAC CGC GGT CTT GAC  
 GAC CAG CGT AAC CCG TAG TTA CGG CAC CGG ATG ATG GCG CCA GAA CTG

480 485 490  
 Val Ser Val Ile Pro Thr Ser Gly Asp Val Val Val Val Ala Thr Asp  
 GTG TCC GTC ATC CCG ACC AGC GGC GAT GTT GTC GTC GTG GCA ACC GAT  
 CAC AGG CAG TAG GGC TGG TCG CCG CTA CAA CAG CAG CAC CGT TGG CTA

Figure 1 (continued)

495					500					505					510
Ala	Leu	Met	Thr	Gly	Tyr	Thr	Gly	Asp	Phe	Asp	Ser	Val	Ile	Asp	Cys
GCC	CTC	ATG	ACC	GGC	TAT	ACC	GGC	GAC	TTC	GAC	TCG	GTG	ATA	GAC	TGC
CGG	GAG	TAC	TGG	CCG	ATA	TGG	CCG	CTG	AAG	CTG	AGC	CAC	TAT	CTG	ACG

515								520				525			
Asn	Thr	Cys	Val	Thr	Gln	Thr	Val	Asp	Phe	Ser	Leu	Asp	Pro	Thr	Phe
AAT	ACG	TGT	GTC	ACC	CAG	ACA	GTC	GAT	TTC	AGC	CTT	GAC	CCT	ACC	TTC
TTA	TGC	ACA	CAG	TGG	GTC	TGT	CAG	CTA	AAG	TCG	GAA	CTG	GGA	TGG	AAG

545						550					555				
Arg	Arg	Gly	Arg	Thr	Gly	Arg	Gly	Lys	Pro	Gly	Ile	Tyr	Arg	Phe	Val
CGT	CGG	GGC	AGG	ACT	GGC	AGG	GGG	AAG	CCA	GGC	ATC	TAC	AGA	TTT	GTG
GCA	GCC	CCG	TCC	TGA	CCG	TCC	CCC	TTC	GGT	CCG	TAG	ATG	TCT	AAA	CAC

560					565					570					
Ala	Pro	Gly	Glu	Arg	Pro	Pro	Gly	Met	Phe	Asp	Ser	Ser	Val	Leu	Cys
GCA	CCG	GGG	GAG	CGC	CCT	CCC	GGC	ATG	TTC	GAC	TCG	TCC	GTC	CTC	TGT
CGT	GGC	CCC	CTC	GCG	GGA	GGG	CCG	TAC	AAG	CTG	AGC	AGG	CAG	GAG	ACA

575					580					585					590
Glu	Cys	Tyr	Asp	Ala	Gly	Cys	Ala	Trp	Tyr	Glu	Leu	Thr	Pro	Ala	Glu
GAG	TGC	TAT	GAC	GCA	GGC	TGT	GCT	TGG	TAT	GAG	CTC	ACG	CCC	GCC	GAG
CTC	ACG	ATA	CTG	CGT	CCG	ACA	CGA	ACC	ATA	CTC	GAG	TGC	GGG	CGG	CTC

				595					600					605		
Thr	Thr	Val	Arg	Leu	Arg	Ala	Tyr	Met	Asn	Thr	Pro	Gly	Leu	Pro	Val	
ACT	ACA	GTT	AGG	CTA	CGA	GCG	TAC	ATG	AAC	ACC	CCG	GGG	CTT	CCC	GTG	
TGA	TGT	CAA	TCC	GAT	GCT	CGC	ATG	TAC	TTG	TGG	GGC	CCC	GAA	GGG	CAC	

Figure 1 (continued)



C20c:

Asn Ser Glu Asn Gln Val Glu Gly Glu Val Gln Ile Val Ser Thr Ala  
 AAT TCG GAA AAC CAA GTG GAG GGT GAG GTC CAG ATT GTG TCA ACT GCT  
 TTA AGC CTT TTG GTT CAC CTC CCA CTC CAG GTC TAA CAC AGT TGA CGA

↑  
 EcoRI

Ala Gln Thr Phe Leu Ala Thr Cys Ile Asn Gly Val Cys Trp Thr Val  
 GCC CAA ACC TTC CTG GCA ACG TGC ATC AAT GGG GTG TGC TGG ACT GTC  
 CGG GTT TGG AAG GAC CGT TGC ACG TAG TTA CCC CAC ACG ACC TGA CAG

↑  
 SfaNI

Tyr His Gly Ala Gly Thr Arg Thr Ile Ala Ser Pro Lys Gly Pro Val  
 TAC CAC GGG GCC GGA ACG AGG ACC ATC GCG TCA CCC AAG GGT CCT GTC  
 ATG GTG CCC CGG CCT TGC TCC TGG TAG CGC AGT GGG TTC CCA GGA CAG

Ile Gln Met Tyr Thr Asn Val Asp Gln Asp Leu Val Gly Trp Pro Ala  
 ATC CAG ATG TAT ACC AAT GTA GAC CAA GAC CTT GTG GGC TGG CCC GCT  
 TAG GTC TAC ATA TGG TTA CAT CTG GTT CTG GAA CAC CCG ACC GGG CGA

Ser Gln Gly Thr Arg Ser Leu Thr Pro Cys Thr Cys Gly Ser Ser Asp  
 TCG CAA GGT ACC CGC TCA TTG ACA CCC TGC ACT TGC GGC TCC TCG GAC  
 AGC GTT CCA TGG GCG AGT AAC TGT GGG ACG TGA ACG CCG AGG AGC CTG

Leu Tyr Leu Val Thr Arg His Ala Asp Val Ile Pro Val Arg Arg Arg  
 CTT TAC CTG GTC ACG AGG CAC GCC GAT GTC ATT CCC GTG CGC CGG CGG  
 GAA ATG GAC CAG TGC TCC GTG CGG CTA CAG TAA GGG CAC GCG GCC GCC

↑  
 NaeI

Gly Asp Ser Arg Gly Ser Leu Val Ser Pro Arg Pro Ile Ser Tyr Leu  
 GGT GAT AGC AGG GGC AGC CTC GTG TCG CCC CGG CCC ATT TCC TAC TTG  
 CCA CTA TCG TCC CCG TCG GAG CAC AGC GGG GCC GGG TAA AGG ATG AAC

Lys Gly Ser Ser Gly Gly Pro Leu Pro Asn  
 AAA GGC TCC TCG GGG GGT CCG CTG CCG AAT TC  
 TTT CCG AGG AGC CCC CCA GGC GAC GGC TTA AG

↑  
 EcoRI

Figure 2

C26d:

Glu Phe Gly Gly Leu Leu Leu Cys Pro Ala Ala Ala Val Gly Ile Phe  
GAA TTC GGG GGC CTG CTG TTG TGC CCC GCG GCA GCC GTG GGC ATA TTT  
CTT AAG CCC CCG GAC GAC AAC ACG GGG CGC CGT CGG CAC CCG TAT AAA

↑  
EcoRI

Arg Ala Ala Val Cys Thr Arg Gly Val Ala Lys Ala Val Asp Phe Ile  
AGG GCC GCG GTG TGC ACC CGT GGA GTG GCT AAG GCG GTG GAC TTT ATC  
TCC CGG CGC CAC ACG TGG GCA CCT CAC CGA TTC CGC CAC CTG AAA TAG

↑  
DdeI

Pro Val Glu Asn Leu Glu Thr Thr Met Arg Ser Pro Val Phe Thr Asp  
CCT GTG GAG AAC CTA GAG ACA ACC ATG AGG TCC CCG GTG TTC ACG GAT  
GGA CAC CTC TTG GAT CTC TGT TGG TAC TCC AGG GGC CAC AAG TGC CTA

Asn Ser Ser Pro Pro Val Val Pro Gln Ser Phe Gln Val Ala His Leu  
AAC TCC TCT CCA CCA GTA GTG CCC CAG AGC TTC CAG GTG GCT CAC CTC  
TTG AGG AGA GGT GGT CAT CAC GGG GTC TCG AAG GTC CAC CGA GTG GAG

↑  
EcoRII

His Ala Pro Arg Ile  
CAT GCT CCC CGA ATT C  
GTA CGA GGG GCT TAA G

↑  
EcoRI

Figure 3



C8h:

Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His Ala  
 CCC TGC ACT TGC GGC TCC TCG GAC CTT TAC CTG GTC ACG AGG CAC GCC  
 GGG ACG TGA ACG CCG AGG AGC CTG GAA ATG GAC CAG TGC TCC GTG CGG

Asp Val Ile Pro Val Arg Arg Arg Gly Asp Ser Arg Gly Ser Leu Leu  
 GAT GTC ATT CCC GTG CGC CGG CGG GGT GAT AGC AGG GGC AGC CTG CTG  
 CTA CAG TAA GGG CAC GCG GCC GCC CCA CTA TCG TCC CCG TCG GAC GAC

Ser Pro Arg Pro Ile Ser Tyr Leu Lys Gly Ser Ser Gly Gly Pro Leu  
 TCG CCC CGG CCC ATT TCC TAC TTG AAA GGC TCC TCG GGG GGT CCG CTG  
 AGC GGG GCC GGG TAA AGG ATG AAC TTT CCG AGG AGC CCC CCA GGC GAC

Leu Cys Pro Ala Gly His Ala Val Gly Ile Phe Arg Ala Ala Val Cys  
 TTG TGC CCC GCG GGG CAC GCC GTG GGC ATA TTT AGG GCC GCG GTG TGC  
 AAC ACG GGG CGC CCC GTG CGG CAC CCG TAT AAA TCC CGG CGC CAC ACG

Thr Arg Gly Val Ala Lys Ala Val Asp Phe Ile Pro Val Glu Asn Leu  
 ACC CGT GGA GTG GCT AAG GCG GTG GAC TTT ATC CCT GTG GAG AAC CTA  
 TGG GCA CCT CAC CGA TTC CGC CAC CTG AAA TAG GGA CAC CTC TTG GAT

↑  
 DdeI

Glu Thr Thr Met Arg Ser Pro Val Phe Thr Asp Asn Ser  
 GAG ACA ACC ATG AGG TCC CCG GTG TTC ACG GAT AAC TCC TC  
 CTC TGT TGG TAC TCC AGG GGC CAC AAG TGC CTA TTG AGG AG

Figure 4

Global Climate Data	
Year	Temperature (°C)
1990	15.2
1991	15.5
1992	15.8
1993	16.1
1994	16.4
1995	16.7
1996	17.0
1997	17.3
1998	17.6
1999	17.9
2000	18.2
2001	18.5
2002	18.8
2003	19.1
2004	19.4
2005	19.7
2006	20.0
2007	20.3
2008	20.6
2009	20.9
2010	21.2
2011	21.5
2012	21.8
2013	22.1
2014	22.4
2015	22.7
2016	23.0
2017	23.3
2018	23.6
2019	23.9
2020	24.2
2021	24.5
2022	24.8
2023	25.1
2024	25.4
2025	25.7
2026	26.0
2027	26.3
2028	26.6
2029	26.9
2030	27.2
2031	27.5
2032	27.8
2033	28.1
2034	28.4
2035	28.7
2036	29.0
2037	29.3
2038	29.6
2039	29.9
2040	30.2
2041	30.5
2042	30.8
2043	31.1
2044	31.4
2045	31.7
2046	32.0
2047	32.3
2048	32.6
2049	32.9
2050	33.2
2051	33.5
2052	33.8
2053	34.1
2054	34.4
2055	34.7
2056	35.0
2057	35.3
2058	35.6
2059	35.9
2060	36.2
2061	36.5
2062	36.8
2063	37.1
2064	37.4
2065	37.7
2066	38.0
2067	38.3
2068	38.6
2069	38.9
2070	39.2
2071	39.5
2072	39.8
2073	40.1
2074	40.4
2075	40.7
2076	41.0
2077	41.3
2078	41.6
2079	41.9
2080	42.2
2081	42.5
2082	42.8
2083	43.1
2084	43.4
2085	43.7
2086	44.0
2087	44.3
2088	44.6
2089	44.9
2090	45.2
2091	45.5
2092	45.8
2093	46.1
2094	46.4
2095	46.7
2096	47.0
2097	47.3
2098	47.6
2099	47.9
2100	48.2

↑  
EcoRI

Phe Ser Gln Met Glu Thr Lys Leu Ile Thr Trp Gly Ala Asp Thr Ala  
TTC TCC CAA ATG GAG ACC AAG CTC ATC ACG TGG GGG GCA GAT ACC GCC  
AAG AGG GTT TAC CTC TGG TTC GAG TAG TGC ACC CCC CGT CTA TGG CGG

Ala Cys Gly Asp Ile Ile Asn Gly Leu Pro Val Ser Ala Arg Arg Gly  
GCG TGC GGT GAC ATC ATC AAC GGC TTG CCT GTT TCC GCC CGC AGG GGC  
CGC ACG CCA CTG TAG TAG TTG CCG AAC GGA CAA AGG CGG GCG TCC CCG

Arg Glu Ile Leu Leu Gly Pro Ala Asp Gly Met Val Ser Lys Gly Trp  
CGG GAG ATA CTG CTC GGG CCA GCC GAT GGA ATG GTC TCC AAG GGT TGG  
GCC CTC TAT GAC GAG CCC GGT CGG CTA CCT TAC CAG AGG TTC CCA ACC

Arg Leu Leu Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu  
AGG TTG CTG GCG CCC ATC ACG GCG TAC GCC CAG CAG ACA AGG GGC CTC  
TCC AAC GAC CGC GGG TAG TGC CGC ATG CGG GTC GTC TGT TCC CCG GAG

Leu Gly Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val  
CTA GGG TGC ATA ATC ACC AGC CTA ACT GGC CGG GAC AAA AAC CAA GTG  
GAT CCC ACG TAT TAG TGG TCG GAT TGA CCG GCC CTG TTT TTG GTT CAC

Glu Gly Glu Val Gln Ile Val Ser Thr Ala Ala Gln Thr Phe Leu Ala  
GAG GGT GAG GTC CAG ATT GTG TCA ACT GCT GCC CAA ACC TTC CTG GCA  
CTC CCA CTC CAG GTC TAA CAC AGT TGA CGA CGG GTT TGG AAG GAC CGT

Thr	Cys	Ile	Asn	Gly	Val	Cys	Trp	Pro	Asn	
ACG	TGC	ATC	AAT	GGG	GTG	TGC	TGG	CCG	AAT	TC
TGC	ACG	TAG	TTA	CCC	CAC	ACG	ACC	GGC	TTA	AG
		↑							↑	
		SfaNI							EcoRI	

3019310

[illegible]

↑  
EcoRI

↑  
HinfI

Thr Phe Thr Ile Glu Thr Ile Thr Leu Pro Gln Asp Ala Val Ser Arg  
ACC TTC ACC ATT GAG ACA ATC ACG CTC CCC CAA GAT GCT GTC TCC CGC  
TGG AAG TGG TAA CTC TGT TAG TGC GAG GGG GTT CTA CGA CAG AGG GCG

Thr	Gln	Arg	Arg	Gly	Arg	Thr	Gly	Arg	Gly	Lys	Pro	Gly	Ile	Tyr	Arg
ACT	CAA	CGT	CGG	GGC	AGG	ACT	GGC	AGG	GGG	AAG	CCA	GGC	ATC	TAC	AGA
TGA	GTT	GCA	GCC	CCG	TCC	TGA	CCG	TCC	CCC	TTC	GGT	CCG	TAG	ATG	TCT

↑                                  ↑  
*Bgl*I                              *Hinf*I

↑  
EcoRI

3019311

C35:

Ile Arg Ser Ile Glu Thr Ile Thr Leu Pro Gln Asp Ala Val Ser Arg  
 ATT CGG TCC ATT GAG ACA ATC ACG CTC CCC CAG GAT GCT GTC TCC CGC  
 TAA GCC AGG TAA CTC TGT TAG TGC GAG GGG GTC CTA CGA CAG AGG GCG

↑  
 EcoRI

Thr Gln Arg Arg Gly Arg Thr Gly Arg Gly Lys Pro Gly Ile Tyr Arg  
 ACT CAA CGT CGG GGC AGG ACT GGC AGG GGG AAG CCA GGC ATC TAC AGA  
 TGA GTT GCA GCC CCG TCC TGA CCG TCC CCC TTC GGT CCG TAG ATG TCT

Phe Val Ala Pro Gly Glu Arg Pro Ser Gly Met Phe Asp Ser Ser Val  
 TTT GTG GCA CCG GGG GAG CGC CCC TCC GGC ATG TTC GAC TCG TCC GTC  
 AAA CAC CGT GGC CCC CTC GCG GGG AGG CCG TAC AAG CTG AGC AGG CAG

↑  
 BglI

Leu Cys Glu Cys Tyr Asp Ala Gly Cys Ala Trp Tyr Glu Leu Thr Pro  
 CTC TGT GAG TGC TAT GAC GCA GGC TGT GCT TGG TAT GAG CTC ACG CCC  
 GAG ACA CTC ACG ATA CTG CGT CCG ACA CGA ACC ATA CTC GAG TGC GGG

Ala Glu Thr Thr Val Arg Leu Arg Ala Tyr Met Asn Thr Pro Gly Leu  
 GCC GAG ACT ACA GTT AGG CTA CGA GCG TAC ATG AAC ACC CCG GGG CTT  
 CGG CTC TGA TGT CAA TCC GAT GCT CGC ATG TAC TTG TGG GGC CCC GAA

Pro Val Cys Gln Asp His Leu Glu Phe Trp Glu Gly Val Phe Thr Gly  
 CCC GTG TGC CAG GAC CAT CTT GAA TTT TGG GAG GGC GTC TTT ACA GGC  
 GGG CAC ACG GTC CTG GTA GAA CTT AAA ACC CTC CCG CAG AAA TGT CCG

Leu Thr His Ile Asp Ala His Phe Leu Ser Gln Thr Lys Gln Ser Gly  
 CTC ACT CAT ATA GAT GCC CAC TTT CTA TCC CAG ACA AAG CAG AGT GGG  
 GAG TGA GTA TAT CTA CGG GTG AAA GAT AGG GTC TGT TTC GTC TCA CCC

Glu Asn Leu Pro Tyr Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg  
 GAG AAC CTT CCT TAC CTG GTA GCG TAC CAA GCC ACC GTG TGC GCT AGG  
 CTC TTG GAA GGA ATG GAC CAT CGC ATG GTT CCG TGG CAC ACG CGA TCC

Ala Gln Ala Pro Pro Pro Ser Trp Asp Gln Met Trp Lys Cys Leu Ile  
 GCT CAA GCC CCT CCC CCA TCG TGG GAC CAG ATG TGG AAG TGT TTG ATT  
 CGA GTT CGG GGA GGG GGT AGC ACC CTG GTC TAC ACC TTC ACA AAC TAA

Arg Leu Lys Pro Thr Leu His Gly Pro Thr Pro Leu Leu Tyr Arg Leu  
 CGC CTC AAG CCC ACC CTC CAT GGG CCA ACA CCC CTG CTA TAC AGA CTG  
 GCG GAG TTC GGG TGG GAG GTA CCC GGT TGT GGG GAC GAT ATG TCT GAC

Gly Ala Ala Glu Phe  
 GGC GCT GCC GAA TTC  
 CCG CGA CGG CTT AAG

↑  
 EcoRI

Figure 7

3019312

C33c:

Glu Phe Gly Ala Val Asp Phe Ile Pro Val Glu Asn Leu Glu Thr Thr  
GAA TTC GGG GCG GTG GAC TTT ATC CCT GTG GAG AAC CTA GAG ACA ACC  
CTT AAG CCC CGC CAC CTG AAA TAG GGA CAC CTC TTG GAT CTC TGT TGG

↑

EcoRI

Met Arg Ser Pro Val Phe Thr Asp Asn Ser Ser Pro Pro Val Val Pro  
ATG AGG TCC CCG GTG TTC ACG GAT AAC TCC TCT CCA CCA GTA GTG CCC  
TAC TCC AGG GGC CAC AAG TGC CTA TTG AGG AGA GGT GGT CAT CAC GGG

Gln Ser Phe Gln Val Ala His Leu His Ala Pro Thr Gly Ser Gly Lys  
CAG AGC TTC CAG GTG GCT CAC CTC CAT GCT CCC ACA GGC AGC GGC AAA  
GTC TCG AAG GTC CAC CGA GTG GAG GTA CGA GGG TGT CCG TCG CCG TTT

Ser Thr Lys Val Pro Ala Ala Tyr Ala Ala Gln Gly Tyr Lys Val Leu  
AGC ACC AAG GTC CCG GCT GCA TAT GCA GCT CAG GGC TAT AAG GTG CTA  
TCG TGG TTC CAG GGC CGA CGT ATA CGT CGA GTC CCG ATA TTC CAC GAT

Val Leu Asn Pro Ser Val Ala Ala Thr Leu Gly Phe Gly Ala Tyr Met  
GTA CTC AAC CCC TCT GTT GCT GCA ACA CTG GGC TTT GGT GCT TAC ATG  
CAT GAG TTG GGG AGA CAA CGA CGT TGT GAC CCG AAA CCA CGA ATG TAC

Ser Lys Ala His Gly Ile Asp Pro Asn Ile Arg Thr Gly Val Arg Thr  
TCC AAG GCT CAT GGG ATC GAT CCT AAC ATC AGG ACC GGG GTG AGA ACA  
AGG TTC CGA GTA CCC TAG CTA GGA TTG TAG TCC TGG CCC CAC TCT TGT

Ile Thr Thr Gly Ser Pro Ile Thr Tyr Ser Thr Tyr Gly Lys Phe Leu  
ATT ACC ACT GGC AGC CCC ATC ACG TAC TCC ACC TAC GGC AAG TTC CTT  
TAA TGG TGA CCG TCG GGG TAG TGC ATG AGG TGG ATG CCG TTC AAG GAA

Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile Ile Ile Cys Asp  
GCC GAC GGC GGG TGC TCG GGG GGC GCT TAT GAC ATA ATA ATT TGT GAC  
CGG CTG CCG CCC ACG AGC CCC CCG CGA ATA CTG TAT TAT TAA ACA CTG

Glu Cys His Ser Thr Asp Ala Thr Ser Ile Leu Gly Ile Gly Thr Val  
GAG TGC CAC TCC ACG GAT GCC ACA TCC ATC TTG GGC ATT GGC ACT GTC  
CTC ACG GTG AGG TGC CTA CGG TGT AGG TAG AAC CCG TAA CCG TGA CAG

Figure 8

Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Val Val Leu Ala Thr  
 CTT GAC CAA GCA GAG ACT GCG GGG GCG AGA CTG GTT GTG CTC GCC ACC  
 GAA CTG GTT CGT CTC TGA CGC CCC CGC TCT GAC CAA CAC GAG CGG TGG

Ala Thr Pro Pro Gly Ser Val Thr Val Pro His Pro Asn Ile Glu Glu  
 GCC ACC CCT CCG GGC TCC GTC ACT GTG CCC CAT CCC AAC ATC GAG GAG  
 CGG TGG GGA GGC CCG AGG CAG TGA CAC GGG GTA GGG TTG TAG CTC CTC

Val Ala Leu Ser Thr Thr Gly Glu Ile Pro Phe Tyr Gly Lys Ala Ile  
 GTT GCT CTG TCC ACC ACC GGA GAG ATC CCT TTT TAC GGC AAG GCT ATC  
 CAA CGA GAC AGG TGG TGG CCT CTC TAG GGA AAA ATG CCG TTC CGA TAG

Pro Leu Glu Val Ile Lys Gly Gly Arg His Leu Ile Phe Cys His Ser  
 CCC CTC GAA GTA ATC AAG GGG GGG AGA CAT CTC ATC TTC TGT CAT TCA  
 GGG GAG CTT CAT TAG TTC CCC CCC TCT GTA GAG TAG AAG ACA GTA AGT

Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Val Ala Leu Gly Ile  
 AAG AAG AAG TGC GAC GAA CTC GCC GCA AAG CTG GTC GCA TTG GGC ATC  
 TTC TTC TTC ACG CTG CTT GAG CGG CGT TTC GAC CAG CGT AAC CCG TAG

Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val Ile Pro Thr  
 AAT GCC GTG GCC TAC TAC CGC GGT CTT GAC GTG TCC GTC ATC CCG ACC  
 TTA CGG CAC CGG ATG ATG GCG CCA GAA CTG CAC AGG CAG TAG GGC TGG

Ser Gly Asp Val Val Val Val Ala Thr Asp Ala Leu Met Thr Gly Tyr  
 AGC GGC GAT GTT GTC GTC GTG GCA ACC GAT GCC CTC ATG ACC GGC TAT  
 TCG CCG CTA CAA CAG CAG CAC CGT TGG CTA CGG GAG TAC TGG CCG ATA

Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr Cys Ala Glu Phe  
 ACC GGC GAC TTC GAC TCG GTG ATA GAC TGC AAT ACG TGT GCC GAA TTC  
 TGG CCG CTG AAG CTG AGC CAC TAT CTG ACG TTA TGC ACA CGG CTT AAG

↑  
 HinfI

↑  
 EcoRI

Figure 8 (Continued)

Diagram illustrating the cloning strategy for the C300 construct:

- C35** (top construct) is digested with *Hinf*I and *Bgl*I to produce fragments **C31** and **C33c**.
- C33c** is digested with *Sfa*NI to produce fragment **C200**.
- C200** is ligated with **C7f** to produce the **C7f+C20c** construct.
- C7f+C20c** is digested with *Dde*I and *Eco*RII to produce fragments **C26d** and **C33c**.
- C26d** and **C33c** are ligated to produce the final **C300** construct.

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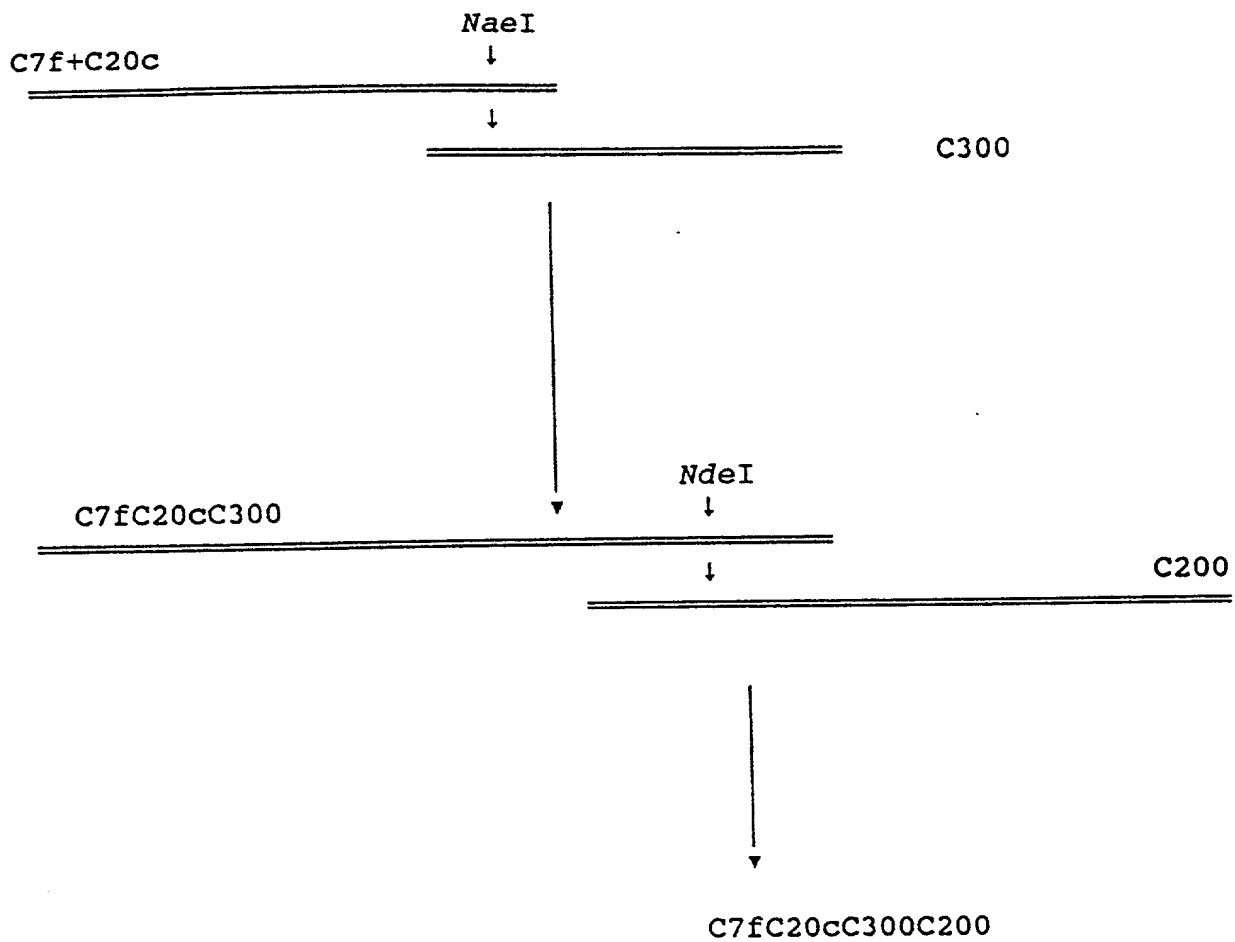


Figure 9 (Continued)



<div style="display: flex; justify-content: space-around;"> <span>-155</span> <span>-150</span> </div> <div> Met Ala Thr Asn Pro Val Cys Val Leu  ATG GCT ACA AAC CCT GTT TGC GTT TTG  TAC CGA TGT TTG GGA CAA ACG CAA AAC </div>															
<div style="display: flex; justify-content: space-around;"> <span>-145</span> <span>-140</span> <span>-135</span> </div> <div> Lys Gly Asp Gly Pro Val Gln Gly Ile Ile Asn Phe Glu Gln Lys Glu  AAG GGT GAC GGC CCA GTT CAA GGT ATT ATT AAC TTC GAG CAG AAG GAA  TTC CCA CTG CCG GGT CAA GTT CCA TAA TAA TTG AAG CTC GTC TTC CTT </div>															
<div style="display: flex; justify-content: space-around;"> <span>-130</span> <span>-125</span> <span>-120</span> <span>-115</span> </div> <div> Ser Asn Gly Pro Val Lys Val Trp Gly Ser Ile Lys Gly Leu Thr Glu  AGT AAT GGA CCA GTG AAG GTG TGG GGA AGC ATT AAA GGA CTG ACT GAA  TCA TTA CCT GGT CAC TTC CAC ACC CCT TCG TAA TTT CCT GAC TGA CTT </div>															
<div style="display: flex; justify-content: space-around;"> <span>-110</span> <span>-105</span> <span>-100</span> </div> <div> Gly Leu His Gly Phe His Val His Glu Phe Gly Asp Asn Thr Ala Gly  GGC CTG CAT GGA TTC CAT GTT CAT GAG TTT GGA GAT AAT ACA GCA GGC  CCG GAC GTA CCT AAG GTA CAA GTA CTC AAA CCT CTA TTA TGT CGT CCG </div>															
<div style="display: flex; justify-content: space-around;"> <span>-95</span> <span>-90</span> <span>-85</span> </div> <div> Cys Thr Ser Pro Gly Pro His Phe Asn Pro Leu Ser Arg Lys His Gly  TGT ACC AGT CCA GGT CCT CAC TTT AAT CCT CTA TCC AGA AAA CAC GGT  ACA TGG TCA GGT CCA GGA GTG AAA TTA GGA GAT AGG TCT TTT GTG CCA </div>															
<div style="display: flex; justify-content: space-around;"> <span>-80</span> <span>-75</span> <span>-70</span> </div> <div> Gly Pro Lys Asp Glu Glu Arg His Val Gly Asp Leu Gly Asn Val Thr  GGG CCA AAG GAT GAA GAG AGG CAT GTT GGA GAC TTG GGC AAT GTG ACT  CCC GGT TTC CTA CTT CTC TCC GTA CAA CCT CTG AAC CCG TTA CAC TGA </div>															
<div style="display: flex; justify-content: space-around;"> <span>-65</span> <span>-60</span> <span>-55</span> </div> <div> Ala Asp Lys Asp Gly Val Ala Asp Val Ser Ile Glu Asp Ser Val Ile  GCT GAC AAA GAT GGT GTG GCC GAT GTG TCT ATT GAA GAT TCT GTG ATC  CGA CTG TTT CTA CCA CAC CGG CTA CAC AGA TAA CTT CTA AGA CAC TAG </div>															
<div style="display: flex; justify-content: space-around;"> <span>-50</span> <span>-45</span> <span>-40</span> <span>-35</span> </div> <div> Ser Leu Ser Gly Asp His Cys Ile Ile Gly Arg Thr Leu Val Val His  TCA CTC TCA GGA GAC CAT TGC ATC ATT GGC CGC ACA CTG GTG GTC CAT  AGT GAG AGT CCT CTG GTA ACG TAG TAA CCG GCG TGT GAC CAC CAG GTA </div>															
<div style="display: flex; justify-content: space-around;"> <span>-30</span> <span>-25</span> <span>-20</span> </div> <div> Glu Lys Ala Asp Asp Leu Gly Lys Gly Gly Asn Glu Glu Ser Thr Lys  GAA AAA GCA GAT GAC TTG GGC AAA GGT GGA AAT GAA GAA AGT ACA AAG  CTT TTT CGT CTA CTG AAC CCG TTT CCA CCT TTA CTT CTT TCA TGT TTC </div>															
<div style="display: flex; justify-content: space-around;"> <span>-15</span> <span>-10</span> <span>-5</span> </div> <div> Thr Gly Asn Ala Gly Ser Arg Leu Ala Cys Gly Val Ile Gly Ile Arg  ACA GGA AAC GCT GGA AGT CGT TTG GCT TGT GGT GTA ATT GGG ATC CGA  TGT CCT TTG CGA CCT TCA GCA AAC CGA ACA CCA CAT TAA CCC TAG GCT </div>															

Figure 10









495					500					505					510
Ala	Leu	Met	Thr	Gly	Tyr	Thr	Gly	Asp	Phe	Asp	Ser	Val	Ile	Asp	Cys
GCC	CTC	ATG	ACC	GGC	TAT	ACC	GGC	GAC	TTC	GAC	TCG	GTG	ATA	GAC	TGC
CGG	GAG	TAC	TGG	CCG	ATA	TGG	CCG	CTG	AAG	CTG	AGC	CAC	TAT	CTG	ACG
				515				520					525		
Asn	Thr	Cys	Val	Thr	Gln	Thr	Val	Asp	Phe	Ser	Leu	Asp	Pro	Thr	Phe
AAT	ACG	TGT	GTC	ACC	CAG	ACA	GTC	GAT	TTC	AGC	CTT	GAC	CCT	ACC	TTC
TTA	TGC	ACA	CAG	TGG	GTC	TGT	CAG	CTA	AAG	TCG	GAA	CTG	GGA	TGG	AAG
			530					535				540			
Thr	Ile	Glu	Thr	Ile	Thr	Leu	Pro	Gln	Asp	Ala	Val	Ser	Arg	Thr	Gln
ACC	ATT	GAG	ACA	ATC	ACG	CTC	CCC	CAA	GAT	GCT	GTC	TCC	CGC	ACT	CAA
TGG	TAA	CTC	TGT	TAG	TGC	GAG	GGG	GTT	CTA	CGA	CAG	AGG	GCG	TGA	GTT
		545					550				555				
Arg	Arg	Gly	Arg	Thr	Gly	Arg	Gly	Lys	Pro	Gly	Ile	Tyr	Arg	Phe	Val
CGT	CGG	GGC	AGG	ACT	GGC	AGG	GGG	AAG	CCA	GGC	ATC	TAC	AGA	TTT	GTG
GCA	GCC	CCG	TCC	TGA	CCG	TCC	CCC	TTC	GGT	CCG	TAG	ATG	TCT	AAA	CAC
	560					565				570					
Ala	Pro	Gly	Glu	Arg	Pro	Pro	Gly	Met	Phe	Asp	Ser	Ser	Val	Leu	Cys
GCA	CCG	GGG	GAG	CGC	CCT	CCC	GGC	ATG	TTC	GAC	TCG	TCC	GTC	CTC	TGT
CGT	GGC	CCC	CTC	GCG	GGA	GGG	CCG	TAC	AAG	CTG	AGC	AGG	CAG	GAG	ACA
575					580					585					590
Glu	Cys	Tyr	Asp	Ala	Gly	Cys	Ala	Trp	Tyr	Glu	Leu	Thr	Pro	Ala	Glu
GAG	TGC	TAT	GAC	GCA	GGC	TGT	GCT	TGG	TAT	GAG	CTC	ACG	CCC	GCC	GAG
CTC	ACG	ATA	CTG	CGT	CCG	ACA	CGA	ACC	ATA	CTC	GAG	TGC	GGG	CGG	CTC
				595				600					605		
Thr	Thr	Val	Arg	Leu	Arg	Ala	Tyr	Met	Asn	Thr	Pro	Gly	Leu	Pro	Val
ACT	ACA	GTT	AGG	CTA	CGA	GCG	TAC	ATG	AAC	ACC	CCG	GGG	CTT	CCC	GTG
TGA	TGT	CAA	TCC	GAT	GCT	CGC	ATG	TAC	TTG	TGG	GGC	CCC	GAA	GGG	CAC

Figure 10 (continued)

			610						615					620			
Cys	Gln	Asp	His	Leu	Glu	Phe	Trp	Glu	Gly	Val	Phe	Thr	Gly	Leu	Thr		
TGC	CAG	GAC	CAT	CTT	GAA	TTT	TGG	GAG	GGC	GTC	TTT	ACA	GGC	CTC	ACT		
ACG	GTC	CTG	GTA	GAA	CTT	AAA	ACC	CTC	CCG	CAG	AAA	TGT	CCG	GAG	TGA		

		625						630					635				
His	Ile	Asp	Ala	His	Phe	Leu	Ser	Gln	Thr	Lys	Gln	Ser	Gly	Glu	Asn		
CAT	ATA	GAT	GCC	CAC	TTT	CTA	TCC	CAG	ACA	AAG	CAG	AGT	GGG	GAG	AAC		
GTA	TAT	CTA	CGG	GTG	AAA	GAT	AGG	GTC	TGT	TTC	GTC	TCA	CCC	CTC	TTG		

		640					645					650					
Leu	Pro	Tyr	Leu	Val	Ala	Tyr	Gln	Ala	Thr	Val	Cys	Ala	Arg	Ala	Gln		
CTT	CCT	TAC	CTG	GTA	GCG	TAC	CAA	GCC	ACC	GTG	TGC	GCT	AGG	GCT	CAA		
GAA	GGA	ATG	GAC	CAT	CGC	ATG	GTT	CGG	TGG	CAC	ACG	CGA	TCC	CGA	GTT		

655						660					665					670	
Ala	Pro	Pro	Pro	Ser	Trp	Asp	Gln	Met	Trp	Lys	Cys	Leu	Ile	Arg	Leu		
GCC	CCT	CCC	CCA	TCG	TGG	GAC	CAG	ATG	TGG	AAG	TGT	TTG	ATT	CGC	CTC		
CGG	GGA	GGG	GGT	AGC	ACC	CTG	GTC	TAC	ACC	TTC	ACA	AAC	TAA	GCG	GAG		

				675					680					685			
Lys	Pro	Thr	Leu	His	Gly	Pro	Thr	Pro	Leu	Leu	Tyr	Arg	Leu	Gly	Ala		
AAG	CCC	ACC	CTC	CAT	GGG	CCA	ACA	CCC	CTG	CTA	TAC	AGA	CTG	GGC	GCT		
TTC	GGG	TGG	GAG	GTA	CCC	GGT	TGT	GGG	GAC	GAT	ATG	TCT	GAC	CCG	CGA		

Figure 10 (continued)